Molecular hydrogen exposure improves functional state of red blood cells in the early postoperative period: a randomized clinical study

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Abstract

Molecular hydrogen (H_2) has been considered a preventive and therapeutic medical gas in numerous diseases. The study aimed to investigate the potential role of molecular hydrogen as a component of anesthesia in surgical treatment with cardiopulmonary bypass (CPB) of acquired valve defects on the functional state of red blood cells (RBC) and functional indicators of cardiac activity. This clinical trial was conducted with 20 patients referring to the Specialized Cardiosurgical Clinical Hospital, Nizhny Novgorod, Russian Federation, who underwent elective surgery with CPB. Twenty-four patients were randomly assigned to two groups. First group included 12 patients (research group) who received H_2 at a concentration of 1.5–2.0% through a facemask using a breathing circuit of the ventilator together with anesthesia immediately after tracheal intubation and throughout the operation. Second group (control group) included 12 patients who were not given H_2. Blood samples were withdrawn from peripheral veins and radial artery at four stages: immediately after the introduction of anesthesia (stage 1), before the start of CPB (stage 2), immediately after its termination (stage 3) and 24 hours after the operation (the early postoperative period) (stage 4). An increase in electrophoretic mobility, an increase in the metabolism of red blood cells, and a decrease in the aggregation of red blood cells relative to the corresponding indicators of the control group were observed in the research group. Patients in the research group had a decrease in oxidative stress manifestations most pronounced one day after the operation. There was a statistically significant difference between the indicators of myocardial contractile function in the research and control group on the 1st and 3rd days after surgery. H_2 inhalation leads to improvement of functional state of red blood cells, which is accompanied by a more favorable course of the early postoperative period. These data show the presence of protective properties of molecular hydrogen.

Key words: erythrocyte; aggregation; molecular hydrogen; malondialdehyde; catalase activity; electrophoretic mobility; diene conjugates; triene conjugates; Schiff bases; lipid peroxidation

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Introduction

Cardiovascular disease is the leading cause of mortality and reduced quality of life.[1] According to the statistics of the World Health Organization, more than 16 million people die from heart diseases every year. Valve cardiac disease accounts for 25% of heart lesions. It is the third-leading global cause of death behind the predominant causes – ischemic heart disease and hypertension.[2] Surgical treatment of acquired valve defects is still the most effective treatment modality for relief of symptoms and heart failure.[3] Valve diseases can only be cured with valve replacement.[4] However, cardiac surgery with cardiopulmonary bypass (CPB) accompanies multidirectional changes at all levels of neuro-reflex regulation, humoral activity, and metabolic status.

The hemorheological factors play an important role in the pathogenesis of acquired valve cardiac diseases. Cardiac surgery has a high rate of postoperative complications such as ischemia/reperfusion injury, inflammatory response, operative tissue trauma, oxidative stress and endothelial dysfunction.[4,5] These factors have both direct and indirect impacts on blood viscosity.[6] Increased blood viscosity is highly dependent on low blood flow in the microcirculation. Red blood cell (RBC) aggregation occurs at low shear rates and increases blood viscosity. Low shear rates can often lead to irregularities in cellular dynamics produced by abnormalities in blood cells, particularly RBCs.[7] The properties of membrane of RBCs are some of the most important determinants of blood rheology and microvascular hemodynamics and influence the shear rates and RBC aggregation.[8]

To preserve their rheological and functional properties, RBCs are equipped with extensive anti-oxidant systems. Recently, experimental studies demonstrated that molecular hydrogen (H_2) is a novel anti-oxidant with certain unique properties. In the last decade, some studies demonstrated that

H_2 reacts with the most cytotoxic of reactive oxygen species.[9,10] Thus, it prevents oxidative stress and effectively protected cells.[9] Since the 2007 discovery that H_2 has selective anti-oxidant properties, multiple studies have shown its therapeutic effects in various human diseases, pathogenesis of which is associated with the toxic effect of reactive oxygen species.[10-12] We have previously shown the anti-oxidant effect of H_2 in cardiac surgery patients.[13] Moreover, it has been reported that H_2 also has anti-inflammatory properties, protective effects against cell death, and anti-allergic effects and stimulates energy metabolism in model animal experiments.[14] Studies of H_2 application in animal model experiments have shown that inhalation of H_2 gas significantly reduces reperfusion brain injury caused by ischemia,[15] or myocardial infarction.[16] Inhalation of 3% H_2 gas for 1 hour twice a day for 7 days in acute cerebral ischemia patients reduces infarct size according to magnetic resonance imaging data and the National Institutes of Health Stroke Scale score.[17]

Pathology of the cardiovascular system is also associated with a violation of membrane cardiomyocytes caused by hypoxia, activation or inhibition of enzyme systems, ultimately leading to excessive lipoperoxidation and accumulation of toxic products during lipid peroxidation processes,[18] which are the cause of secondary damage to organs and tissues.[19,20] Therefore, the relevance of studying the process of lipid peroxidation processes and its correction in cardiac patients is not in doubt. H_2 reduces oxidative stress by direct reactions with strong oxidants,[21] but also indirectly by regulating the expression of various genes, exerting a multifaceted effect on the processes of inflammation,[22] apoptosis,[23] and metabolism.[24]

H_2 has several therapeutic benefits compared to other known antioxidants. First, its small molecular size allows it to penetrate any biological membrane, including the mitochondria, where H_2 suppresses cytotoxic free radicals at the site of their formation, and into the nucleus, where H_2 prevents the oxidative destruction of DNA. Secondly, H_2 selectively reacts with free radicals, it neutralizes mainly cytotoxic radicals (OH^- and $ONOO^-$), without affecting less active signaling molecules.[25] In addition, H_2 is a conditionally inert substance with zero redox potential in aqueous solutions; it does not exhibit toxic properties even under prolonged exposure in the form of highly concentrated gas mixtures.[26]

Based on these previous studies, the purpose of the study was to investigate the potential effects of H_2 as a component of the anesthesia in surgical treatment with CPB of acquired valve defects by improving the electrokinetic, aggregation, metabolic and anti-oxidant parameters of RBCs.

Subjects and Methods

Study design

This study follows the CONsolidated Standards Of Reporting Trials (CONSORT) statement for protocol reporting (**Additional file 1**). The sample size was calculated by considering the study power of 80% and a type 1 error of 5% and the minimum expected difference in functional state of RBC score (electrophoretic mobility) between research and control groups. Forty patients with heart failure acquired valvar cardiac disease were identified in the database of the 3rd cardiac surgery department of the Specialized Cardiosurgical Clinical Hospital, Nizhny Novgorod, Russian Federation. Of these, 6 patients were < 10 years, 3 had complex-associated congenital heart defects (corrected aortic dextraposition, atrial septal defect, abnormal confluence of the pulmonary veins), 7 refused to participate in the study, and 4 were lost in follow-up (did not undergo CPB). Some patients met multiple exclusion criteria. Twenty patients remained (16 males, 4 females; mean age 57.6 ± 7.8 years) who underwent elective surgery with CPB in Specialized Cardiosurgical Clinical Hospital, Nizhny Novgorod, Russian Federation (**Figure 1**).

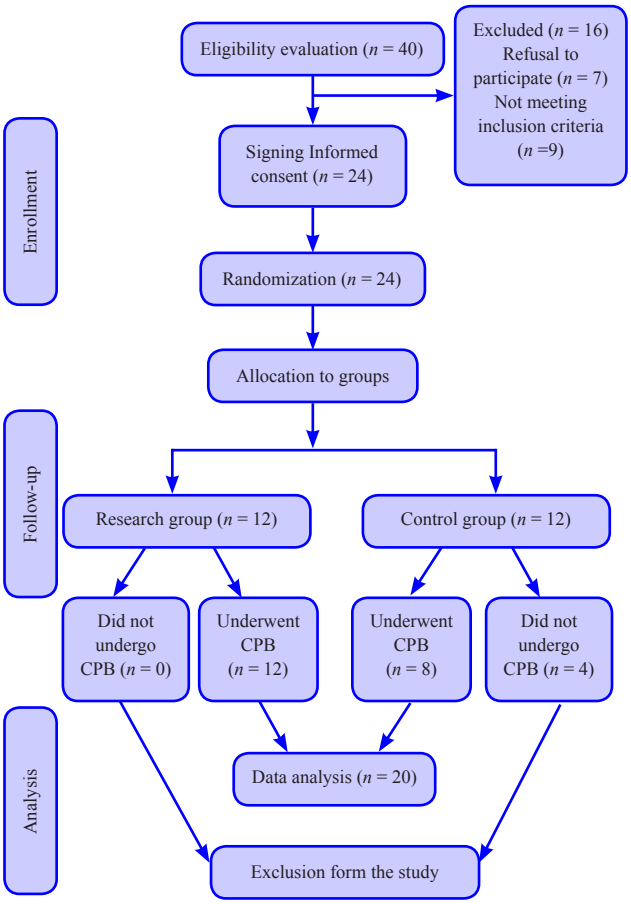

Figure 1: The flow chart.
Note: CPB: cardiopulmonary bypass.

Selection criteria

A total of 20 patients of either sex were included. There were no significant differences between patients from the two groups (**Table 2**). The severity of the patient's condition was determined according to the classification of the New York Heart Association,[27] and patients belonging to III and IV functional class were included (**Table 2**).

Patients with prior history of allergy to treatment drugs, psychological problems, pregnancy, and no lack of patient cooperation before surgery were exempted from the study.

Preoperative preparation and anesthesia

Preanesthetic evaluation was done a day before elective sur-

Table 1: Dynamics of diene and triene conjugates and Schiff's bases in patients with CPB (relative units)

	Stage 1	Stage 2	Stage 3	Stage 4
Research group				
Diene conjugates				
Arterial	1.365±0.511	1.87±0.457	2.13±0.369#	1.987±0.764
Venous	1.797±0.454	2.774±0.618	1.773±0.905	1.648±0.438
Triene conjugates				
Arterial	2.027±0.333	0.756±0.281#	4.492±1.007#	1.459±0.479
Venous	0.906±0.569	0.992±0.615	0.720±0.294	0.657±0.407
Schiff's bases				
Arterial	3.261±0.568	2.325±0.692#	5.616±1.771	2.597±0.396#
Venous	1.171±0.493	1.243±0.405	0.810±0.352	0.972±0.525
Control group				
Diene conjugates				
Arterial	1.413±0.566	1.612±0.344	1.475±0.455	1.589±0.265
Venous	3.152±0.544	2.053±1.067	1.545±0.375#	1.775±0.845#
Triene conjugates				
Arterial	0.768±0.227	0.903±0.265	0.807±0.235	0.622±0.253
Venous	1.165±0.879	0.857±0.389	1.069±0.482	1.308±0.694
Schiff's bases				
Arterial	0.84±0.291	1.262±0.116#	1.042±0.462	1.167±0.361
Venous	1.032±0.242	0.850±0.463	0.969±0.381	1.655±0.217#

Note: Data are expressed as mean ± SD (n = 12 in research group, who were given anesthesia with inhalation of molecular hydrogen; n = 8 in control group, who received standard cardiopulmonary bypass). #P < 0.05, *vs.* control group (Student's t-test). 2,3-DPG: 2,3-Diphosphoglyceric acid; ATP: adenosine triphosphate; CPB: cardiopulmonary bypass; stage 1: immediately after the introduction of anesthesia; stage 2: before the start of CPB; stage 3: immediately after its termination; stage 4: 24 hours after the operation.

gery along with routine investigations. Patients kept nothing by mouth for 10 hours and then were taken to the operating room the following morning. All patients received standard premedication with Diazepam (Relanium, "Polfa Tarchomin S.A.", Warsaw, Poland) at a dose of 0.15 mg/kg intramuscularly 30 minutes before surgery. Induction of anesthesia performed with a combination of diazepam at a dose of 0.2–0.3 mg/kg and propofol (Lipuro B.Braun, Melsungen Germany) at a dose of 2 mg/kg. Maintenance of anesthesia was achieved by inhaling 1–4% sevoflurane (Sevoran, Abbot, Queenborough, UK) in air-oxygen mixture (50% of oxygen). Fentanyl was applied as an additional analgesic component. Myoplegia was achieved by arduan (Gedeon Richter, Budapest, Hungary) at a dose of 0.1 mg/kg.

Sample randomization and H_2 inhalation

The study enrolled 20 patients who underwent planned surgery with CPB in theSpecialized Cardiosurgical Clinical Hospital, Nizhny Novgorod, Russian Federation in the period from September 2018 to April 2019. All patients were divided into two groups using a computer random number generator. First (research) group included 12 patients who had inhalation H_2 using a ventilator circuit together with anesthesia. The H_2 was obtained using a hydrogen generator "Boson-N H_2" (NPP "ECONIKA", Odessa, Ukraine). Patients inhaled 1.5–2% H_2 through a facemask using a ventilator circuit immediately after tracheal intubation and throughout the operation. Second (control) group included 12 patients who did not receive H_2. All the necessary data was collected and recorded by a senior medical student who did not report on the patient groups; also, the patients did not know about the research group they were in. Molecular hydrogen was prepared by an anesthesiologist, and anesthesia was performed by a resident.

Measurements

The primary outcome of this study was the functional state of RBC score; the safety endpoints included an assessment of changes in electrophoretic mobility of RBC, erythrocyte aggregation, and concentration of adenosine triphosphate (ATP) and 2,3-diphosphoglyceric acid (2,3-DPG). The secondary efficacy outcomes included the content of Schiff bases content of diene, triene conjugates and Schiff bases and malondialdehyde (MDA) concentration, catalase activity score to assess intensity of lipid peroxidation processes.

As tertiary outcomes, we examined the character of recovery of cardiac activity after cardioplegia. Cardiac geometry and function were evaluated using the General Electric Vivid 7 Dimension Ultrasonic Scanner (Vingmed Ultrasound AS, Horten, Norway), a sensor with a frequency of 3.5 MHz from the 1st (postoperative), 2nd and 3rd day after the operation. Following the live data collection, end diastolic volume, left ventricular systolic volume, and left ventricular fractional shortening biplane method of disks (modified Simpson method[28]) were reviewed.

The following indicators were used for a comprehensive comparative assessment of RBC functional state.

We withdraw blood samples from their peripheral veins and radial artery at four stages: immediately after the introduction of anesthesia (stage 1), before the start of CPB (stage 2) and immediately after its termination (stage 3) and 24 hours after the operation (stage 4).

Electrophoretic mobility (EPM) of RBC, their aggrega-

tion, concentration of ATP, 2,3-DPG, diene conjugates, triene conjugates, Schiff's bases, MDA and catalase activity in RBC were studied.

We measured the EPMs of human RBC using the microelectrophoresis (analytical complex "Cito-Expert" (TU 9443-137-43674401-2005), NTU Engineering and Technical Center, Izhevsk, Russian Federation) in our modification.[29] We diluted a suspension of washed erythrocytes with 10 mM Tris-HCl phosphate-buffered saline of pH 7.4 and registered the time of passage of the distance of 100 μm by RBC in the Tris-HCl phosphate-buffered saline (pH 7.4) at a current strength with an intensity of 12 mA.

EPM was calculated as the following: $U = S/TH$

Where U is the EPM, S is the migrated distance, T is time taken for the migration and H is potential gradient.

Potential gradient (H) was measured using the following equation: $H = I/g\chi$

Where I is current strength, g is cross section of the camera, and χ is the specific conductivity of the medium.

Erythrocyte aggregation was studied using optical microscopy (Meiji Techno Co., Ltd., Saitama, Japan) by counting single RBC and their aggregates.[30] To stimulate aggregation, we used a solution of blue dextran T-2000 (20 mg/mL, GE Healthcare, Moscow, Russian Federation) in a Tris-hydrochloric acid buffer (pH 7.4). The washed erythrocytes were diluted with a solution of blue dextran (in a ratio of 1:10 by volume) and the number of non-aggregated RBCs was counted in Goryaev's camera (LLC Minimed, Bryansk, Russian Federation). The total number of RBC in the sample was calculated in isotonic NaCl solution. Aggregation index was calculated as the following: Aggregation index (%) = 100 – (the number of non-aggregated (free) RBCs / all RBCs × 100).

The concentrations of ATP and 2,3-DPG were determined using a non-enzymatic method in trichloroacetic acid (TCA) filtrate of hemolyzed RBCs.[31] The washed erythrocytes (1 mL) were suffer hemolyzed by cold distilled water (2 mL) for 20 minutes, proteins were deposited with two volumes of 12% TCA, and the precipitate was separated and centrifuged at 100 × g for 15 minutes. Then, we removed the supernatant liquid through a paper filter. TCA-filtrate of hemolyzed erythrocytes was used to determine ATP and 2,3-DPG. To evaluate the ATP 1 mL of 2 M hydrochloric acid (HA) was added to the TCA filtrate. The hydrolysis was carried out in a boiling water bath for 7 minutes, and then was cooled and neutralized with an equal volume 2 M NaOH. We determined inorganic phosphorus (Pi), which included Pi that had split off from ATP after hydrolysis, and Pi before hydrolysis.[32]

To determine 2,3-DPG, nucleotides (ATP, adenosine diphosphate, adenosine monophosphate) were removed from the TCA-filtrate of hemolyzed RBC by adsorption on activated carbon followed by centrifugation. In the supernatant (0.5 mL), Pi1 was determined. Part of the TCA-filtrate (0.5 mL) was subjected to ashing by adding 0.5 mL of a 5% solution of magnesium nitrate, boiled. After cooling, the contents of the test tube were dissolved in 0.5 mL of 0.36 N H_2SO_4. In the supernatant (0.5 mL), Pi2 was determined. The Pi was determined by registering the color density on the photoelectric photometer KFK-3 -"ZOMZ" (JSC ZOMZ, Sergiev Posad, Russian Federation) at a wavelength of 660 nm. The Pi concentration was determined using a calibration curve using a standard KH_2PO_4 solution. The concentration of 2,3-DPG was calculated as the following: (Pi1 × 100 – Pi2 × 10) / 2.

Lipid peroxidation

The intensity of lipid peroxidation processes was studied by the content of diene, triene conjugates, Schiff's bases, determining their content in plasma by the method of I. A. Volchegorsky.[33] Phase separation was carried out in a heptane-isopropanol mixture. Optical densities (E) were measured using an SF-2000 spectrophotometer (CJSC OKB Spectrum, St. Petersburg, Russian Federation), evaluating each phase at wavelengths of 220 nm (absorption of broken double bonds), 232 nm (absorption of diene conjugates), 278 nm (absorption of triene conjugates), and 400 nm (absorption of Schiff's bases). The content of diene, triene conjugates and Schiff bases was calculated from the relative values of E232\E220, E278\E220, E400\E220 and in relative units.

The MDA concentration was determined by reaction with 2-thiobarbituric acid to form a colored trimethylene complex with a maximum absorption at 530 nm.[34] The MDA concentration was measured using the molar extinction coefficient: E = 1.56×10^{-5} $M^{-1} \cdot cm^{-1}$.

Catalase activity was analyzed according to the reduction of peroxide.35 We then carried out spectrophotometrically immediately and 20 seconds after H_2O_2 was added to the sample cell at a wavelength of 240 nm. Catalase activity (A) was measured using the following equation: A = (lg E1/E2 × 120,000)/Hb.

where E1, E2 – the molar extinction coefficient of the test sample immediately and 20 seconds after adding H_2O_2, Hb – concentration hemoglobin in the test.[35] For the catalase activity of RBC, the amount of μmol of H_2O_2 converted by the enzyme into a unit of time was calculated per mg of hemoglobin in the sample, i.e., μmol/min × mgHB.

Ethical consideration

The study was approved by the Local Ethical Committee of Specialized Cardiosurgical Clinical Hospital (Protocol No. 2; on February 19, 2019) (**Additional file 2**) and complies with the *Declaration of Helsinki*, and the requirements set out in the main regulatory documents of the Russian Federation on clinical research. Written informed consent (**Additional file 3**) was obtained from each patient before participation in the study.

Statistical analysis

The obtained data were processed using BIOSTAT (AnalystSoft Inc., Walnut, CA, USA) and Microsoft Excel for Windows (MS Office 2016 (16.0.5266.1000), MSO (16.0.5266.1000), Version 64, Santa Rosa, CA, USA) application software packages using one-dimensional statistics methods. To test the hypothesis about the type of distribution, the Shapiro-Wilk method was used. The study of statistical patterns in the samples was carried out using a parametric criterion (Student's t-test), since the normal distribution of features was shown. Parameters such as the arithmetic mean of the sample population and the standard deviation according to the Student's criterion were calculated. The differences were considered significant at a significance level of $P < 0.05$.

Results

Studies of erythrocyte aggregation and EPM have shown an increase in EPM and a decrease in aggregation of arterial blood one day after the operation in the research group. Erythrocyte aggregation of venous blood did not change at all stages of the study, although the EPM increased relative to the baseline level (**Figure 2**). Control group on the contrary the EPM decreased at 2nd and 3rd stages of the study and was restored to its original level by 1 day after operation. The EPM of venous blood did not change at all stages of the study. The erythrocyte aggregation decreased only at the 3rd stage of the study and increased relative to the initial level at the 3rd and 4th stages of the study.

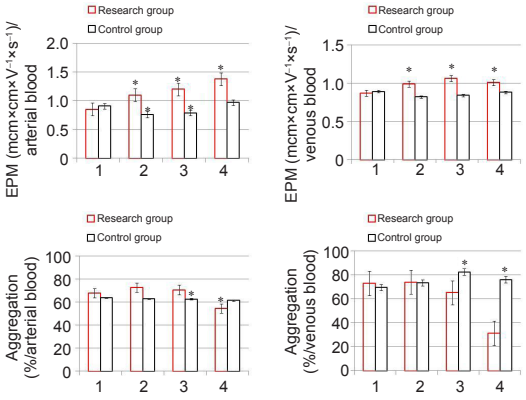

Figure 2: Dynamics of EPM and erythrocyte aggregation in patients with CPB.
Note: Data are expressed as mean ± SD (n = 12 in research group, who were given anesthesia with inhalation of molecular hydrogen; n = 8 in control group, who received standard cardiopulmonary bypass). *P < 0.05, *vs.* stage 1 (Student's *t*-test). CPB: cardiopulmonary bypass; EPM: Electrophoretic mobility; stage 1: immediately after the introduction of anesthesia; stage 2: before the start of CPB; stage 3: immediately after its termination; stage 4: 24 hours after the operation.

In addition, our results indicate a significant increase in ATP concentration in arterial blood erythrocytes at the 3rd and 4th stages in the research group (**Figure 3**). The concentration of 2,3-DPG in arterial blood RBC in this group increased at stage 2 and remained elevated until the end of the study. Significant changes in these indicators in venous blood in the research group were not observed. In the control group, changes in the concentration of ATP were shown in arterial blood at stages 3 and those in the concentration of 2,3-DPG were shown in arterial blood at stages 3 and 4. At the same time, a decrease in the concentration of 2,3-DFG in arterial blood erythrocytes was recorded at stage 2 and a decrease in the concentration of ATP in venous blood erythrocytes was recorded at stage 3 (**Figure 3**).

Analysis of oxidative processes revealed a statistically significant (P < 0.05) decrease in the number of triene conjugates and Schiff's bases before the start of artificial blood circulation and a day after surgery. There was also an increase in the content of diene conjugates and triene conjugates after the end of artificial blood circulation relative to the first stage of the study in patients of the research group. Analysis of venous blood samples showed a statistically significant increase in the diene conjugates level before the start of artificial blood circulation (P < 0.05), which was restored by the 3rd stage of the study. At the same time, after the end of

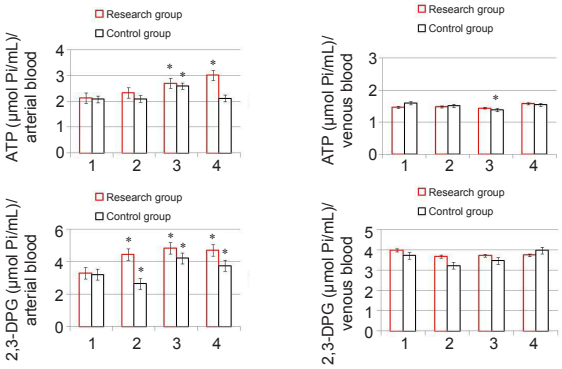

Figure 3: Dynamics of ATP and 2,3-DPG concentrations in erythrocytes of patients with CPB.
Note: Data are expressed as mean ± SD (n = 12 in research group, who were given anesthesia with inhalation of molecular hydrogen; n = 8 in control group, who received standard cardiopulmonary bypass). *P < 0.05, *vs.* stage 1 (Student's *t*-test). 2,3-DPG: 2,3-diphosphoglyceric acid; ATP: adenosine triphosphate; CPB: cardiopulmonary bypass; stage 1: immediately after the introduction of anesthesia; stage 2: before the start of CPB; stage 3: immediately after its termination; stage 4: 24 hours after the operation.

artificial blood circulation, there was a tendency to decrease triene conjugates and Schiff's bases. This trend continued a day after the operation. In the control group, a statistically significant (P < 0.05) increase in Schiff's bases concentration in arterial blood samples was registered during the study relative to the first stage. The number of triene conjugates and Schiff's bases in venous blood samples increased a day after the operation (**Table 1**).

The study compared the activity of pro-and anti-oxidant processes between the groups and revealed that when using H_2, the concentration of MDA in arterial RBCs decreased relative to the initial level from the 3rd stage of the study. In the control group, the concentration of MDA increased a day after the operation and did not significantly differ from the initial level. In venous blood, the changes of these processes were multidirectional: a decrease in the concentration of MDA was observed in the research group by the stage 4 and an increase in the indicator relative to the initial level at the 3rd stage in the control group. The catalase activity of arterial blood in the research group also had a multidirectional character: the catalase activity decreased in the research group and increased in the control group. In venous blood, changes in catalase activity were characterized by its growth: at the 3rd stage in the research group and at the 4th stage in the control group relative to the initial values (**Figure 4**).

In our study, we have found that using H_2 as a component of the anesthesia in surgical treatment with CPB of acquired valve defects result in increase in EPM of human RBC, decrease in erythrocyte aggregation, concentration of MDA, the catalase activity and increase in enhancing erythrocyte metabolism. The obtained results of the functional state of RBC were combined with the dynamics of the functional indicators of the heart. A beneficial effect of recovery of cardiac activity after cardioplegia was attributed to independent recovery of heart contractions or after a single heart defibrillation. The present study shows a beneficial type of heart rate recovery after opening the aorta was more common in the research group, which was reliably significant (**Figure 5**).

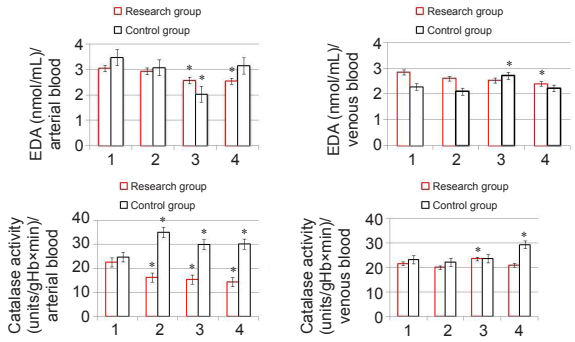

Figure 4: Dynamics of MDA concentration and catalase activity in patients with CPB.
Note: Data are expressed as mean ± SD (n = 12 in research group, who were given anesthesia with inhalation of molecular hydrogen; n = 8 in control group, who received standard cardiopulmonary bypass). *P < 0.05, *vs.* stage 1 (Student's t-test). CPB: cardiopulmonary bypass; MDA: malondialdehyde; stage 1: immediately after the introduction of anesthesia; stage 2: before the start of CPB; stage 3: immediately after its termination; stage 4: 24 hours after the operation.

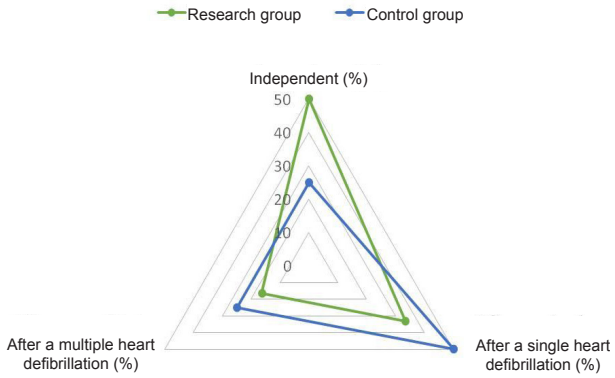

Figure 5: The character of recovery of cardiac activity after cardioplegia.
Note: Plotted data represent two groups (n = 12 in research group, who were given anesthesia with inhalation of molecular hydrogen; n = 8 in control group, who received standard cardiopulmonary bypass). Data are expressed as a percentage of patients in the corresponding group.

Average time of CPB was 74.3 ± 25.8, and 76.1 ± 27.3 minutes in research and control groups, respectively. Average time of a cross-clamp on the aorta was 67.2 ± 18.2 and 60.2 ± 13.2 minutes in research and control groups respectively. Thus, there wereno significant differences in age, sex, the severity of the condition, the nature of the surgical intervention, the time of artificial circulation, or the time of aortic compression between two groups (**Table 2**).

Table 2: Clinical characteristics of patients with CPB

Parameters	Research group (n=12)	Control group (n=8)
New York Heart Association Class (IV/ III)[a]	3/9	2/6
Age (yr)[b]	57.1±6.3	62.3±5.5
Sex (female/male)[a]	2/10	2/6
Duration of CPB (min)[b]	74.3±25.8	76.1±27.3
Duration of aortic cross-clamping (min)[b]	67.2±18.2	60.2±13.2

Note: [a]Data are expressed as number and [b]Data are expressed as mean ± SD. Research group received anesthesia with inhalation of molecular hydrogen; control group received standard cardiopulmonary bypass. CPB: cardiopulmonary bypass.

The indicators of myocardial contractile function in the research group on the 1st and 3rd days after surgery were significantly higher than those in the control group (**Table 3**). Thus, the left ventricular fractional shortening was increased by 7% on the 1st day and by 20% on the 3rd days relative to this indicator in the control group. End diastolic volume and left ventricular systolic volume showed a decrease in the research group from the first day after the operation and changed significantly on the 2nd and 3rd days after the operation.

Table 3: Functional indicators of cardiac activity in patients with cardiopulmonary bypass

Day after operation	Left ventricular fractional shortening (%)	End diastolic volume/body surface area (mL/m^2)	Left ventricular systolic volume/ body surface area (mL/m^2)
Research group			
1	55.2±6.0	143.2±17.0	78.3±18.3
2	57.4±5.9†	135.4±19.1†	78.3±24.7†
3	58.4±4.9†	120.0±8.2†	62.0±13.0
Control group			
1	51.4±6.8	162.2±15.6	85.3±12.2
2	47.7±4.9	160.7±13.1	102.3±18.5
3	48.6±6.3	109.3±9.2	72.0±17.6

Note: Data are expressed as mean ± SD (n = 12 in research group, who were given anesthesia with inhalation of molecular hydrogen; n = 8 in control group, who received standard cardiopulmonary bypass). †P < 0.05, *vs.* 1 day after operation (Student's t-test).

Discussion

Discussing the effect of H_2, it should be noted the effect of H_2 on the processes of lipoperoxidation, as well as on the metabolism of red blood cells. Recent studies reported in the literature indicated that ventilation with H_2 significantly increased the activity of ATP synthase.[36] In our studies, there was an increase in the ATP concentration in the metabolic processes of RBCs under the action of H_2 , which indicates an improvement in the metabolism of RBCs and is probably due to the restoration of redox processes, a decrease in intermediate metabolic products, including lactic acid, and a decrease in acidosis in cells. In particular, it has been shown that H_2 can effectively reduce oxidation caused by OH-.[37] In addition, H_2 suppresses chain reactions that develop under oxidative stress, which leads to lipid peroxidation and to an increase in the concentration of markers such as 4-hydroxyl-2-nonenal and MDA.[38]

Our study revealed a more pronounced and prolonged decrease in the concentration of MDA under the action of H_2 in cardiac surgery patients compared with the control group. The reduction of lipoperoxidation processes under the action of H_2 may be an important factor determining the increase in the stability of erythrocyte membranes and the improvement of their aggregation properties.

The concentration of ATP and 2,3-DPG also affects the deformation and aggregation of RBCs. Thus, ATP serves as a phosphate donor for protein kinase reactions that phosphorylate membrane proteins, which increases the deformation of erythrocytes. Increasing the concentrations of ATP in RBCs

leads to phosphorylation of spectrin, ankyrin, protein 4.1R, and weakens protein-protein interactions.[39] In that way there may be a role for the ATP to increase the deformation of RBC, affecting their aggregation.[40] In addition, the growth of 2,3-DPG is accompanied by dissociation of the spectrum, an increase in the integral mobility of proteins of the erythrocyte membrane, an increase in density and a decrease in cell volume.[41]

It should be noted that there was significant difference in the concentration of ATP in arterial and venous blood between the group using H_2 and the control group 1 day after the operation. This may indicate the release of ATP from RBC during their deformation in the microcirculation. The mechanical deformation of RBCs has been reported to stimulate the release of ATP[42] and nitric oxide from these cells.[43] ATP released from RBCs helps regulate vascular tone by binding with purigenic receptors in endothelial cells, which then respond by releasing nitric oxide, a potent vasodilator, into the surrounding smooth muscle cells.[44] Changes in vascular tone affect the intensity of blood flow. A long-term increase in erythrocyte aggregation is accompanied by a chronic decrease in nitric oxide synthase expression in endothelial cells. Thus, high-intensity erythrocyte aggregation can lead to a decrease in the severity of compensatory vasodilator reactions.[45] It is important to note that the changes in the studied parameters of RBC revealed in our work were recorded not only with the direct use of H_2, but also in the postoperative period. This fact suggests an indirect effect of H_2.

Probably, H_2 is a regulatory molecule involved in many cell functions, both for intracellular and, perhaps less well known, extracellular functions. For example, an increase in the level of catecholamines in blood plasma is associated with increased aggregation of RBC due to the activation of erythrocyte adrenoreceptors.[46] It is assumed that H_2 leads to reduction in stress response and in catecholamines, thus resulting in a decrease in the aggregation of RBC. In our previous studies,[26,34,47] it was revealed that a decrease in EPM is associated with an increase in adrenoreactivity and the development of the first phase of stress, while an increase in EPM is observed with the development of the second phase of stress reaction and is due to an increase in glucocorticoids and resistance of the body.[47] As shown in this study, the currently used H_2 leads to increase in EPM, which may indicate a decrease in the stress reaction of cardiac surgery patients to an operating injury.

There were some limitations in this study. The number of patients included in most clinical trials was insufficient. Patients received H_2 for a short period of time. Thus, the duration of administration and the total number of H_2 inhalation sessions for a full assessment of the effect should be determined. Most patients were followed up only for a short period after treatment. Therefore, the long-term efficacy and effect of H_2 on the course of the disease are still unclear.

In any case, using H_2 as a component of the anesthesia in surgical treatment with cardiopulmonary bypass of acquired valve defects leads to the improvement in functional state of RBC, which is accompanied by a more favorable course in the early postoperative period. These data indicate the protective properties of H_2. Subsequent studies will address this possibility.

Author contributions
YDB, VVP, APM: Study design, fund acquisition; AVD, DAD, EIN: manuscript writing, data analysis and reference literature search, data interpretation; EVT, SAF, AYS, EVM: data acquisition and analysis. All authors approved the final manuscript for publication.

Conflicts of interest
EIN is an employee of Limited Liability Company "Research and production enterprise" Ekonika. There is no conflict of interest.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Additional files
Additional file 1: CONSORT checklist.
Additional file 2: Hospital Ethics Approval (Russian).
Additional file 3: Informed consent form.

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